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# The importance of initial application and reapplication of biochar in the context of soil structure improvement

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**Abstract:** It was shown that the use of biochar provides many benefits to agriculture by improving the whole complex of soil properties, including soil structure. However, the diverse range of biochar effects depends on its physicochemical properties, its application rates, soil initial properties etc. The impacts of biochar, mainly its reapplication to soils and its interaction with nitrogen in relation to water-stable aggregates (WSA) did not receive much attention to date. The aims of the study were: (1) to evaluate the effect of initial application (in spring 2014) and reapplication (in spring 2018) of different biochar rates (B0, B10 and B20 t $ha^{-1}$) as well as application of biochar with N-fertilizer (40 to 240 kg N $ha^{-1}$ depending on the requirement of the cultivated crop) on the content of WSA as one of the most important indicators of soil structure quality, (2) to assess the interrelationships between the contents of soil organic matter (SOM) and WSA. The study was conducted in 2017–2019 as part of the field experiment with biochar on Haplic Luvisol at the experimental station of SUA in Nitra, Slovakia. Results showed that initial application as well as reapplication of biochar improved soil structure. The most favorable changes in soil structure were found in N0B20B treatment (with biochar reapplication) at which a significantly higher content of water-stable macro-aggregates ($WSA_{ma}$) (+15%) as well as content of $WSA_{ma}$ size fractions of > 5 mm, 5–3 mm, 3–2 mm and 2–1 mm (+72%, +65%, +57% and +64%, respectively) was observed compared to the control. An increase in SOM content, due to both, initial biochar application and its reapplication, significantly supported the stability of soil aggregates, while organic matter including humic substances composition did not.

**Keywords:** Biochar; Soil organic matter; Water-stable aggregates; Soil structure; Haplic Luvisols.

## INTRODUCTION

Structure is one of the basic physical soil characteristics, which controls a variety of physical, chemical and biological processes in soils (Bronick and Lal, 2005; Czachor and Lichner, 2013; Leelamanie and Karube, 2014; Shukla, 2014). Soil structure is usually defined as spatial arrangement of soil organic substances, mineral particles and the pores between them (Fiedler and Reissing, 1964; Rząsa and Owczarzak, 2004). Soil structure can also be described as an arrangement of soil particles into larger formations of different shapes and sizes called aggregates (Pires et al., 2017). The soil with good structure has low compaction or bulk density and a large amount of pore space. These soils have high infiltration rate, quick water movement through the profile, high water retention, high water availability to roots, low crusting on soil surface, high gas exchange, high nutrient availability, easier root penetration, reduced surface runoff and soil erosion intensity (Shukla, 2014). Soil structure is dynamic complex which is not very well understood despite numerous advances in clay mineralogy, colloidal chemistry and other scientific areas which have since led to a better understanding of genesis, characterization, and management of soil structure (Carter and Stewart, 1996). Formation of aggregates in the soils is one of the most important problems of soil science. Formation of soil aggregates as basic unit of pedal soil structure is a function of physical forming forces (such as: inter and intramolecular forces, electrostatic, and gravitational forces) between soil particles (Grosbellet et al., 2011; Hu et al., 2015) however, its stabilization is influenced by internal and external factors, and their interactions (Bogunovic et al., 2020; Jozefaciuk and Czachor, 2014; Paradello et al., 2013).

As it was mentioned above, an aggregate is the basic unit of soil structure. Based on their size, soil aggregates are divided into two groups: – macro-aggregates (> 250 μm) and micro-aggregates (< 250 μm). The aggregates resisting the action of water are called water-stable aggregates (Fulajtár, 2006). The higher content of water-stable aggregates, the better and more stable the soil structure (Šimanský et al., 2018b). Soil organic matter (SOM) is one of the most important internal factors affecting soil structure (Bronick and Lal, 2005; Tisdall and Oades, 1982), since SOM acts as a significant binding agent for soil particles (Bronick and Lal, 2005). SOM affects the water stability of aggregates by decreasing their wettability and increasing their mechanical strength (Onweremadu et al., 2007). It is already well known that soil management practices influence the content and quality of SOM (Beck-Broichsitter et al., 2020; Leelamanie and Manawardana, 2019), which is a very important factor of water-stable aggregates (WSA) formation (Onweremadu et al., 2007).

SOM content and the stability of soil aggregates are two important soil attributes that are interconnected and interdependent. They are accounted as significant indicators of soil quality and degradation (Czachor and Lichner, 2013; Gaida et al., 2013; Leelamanie and Karube, 2014; Shukla, 2014). The breakdown of soil macro-aggregates can be followed by a release of SOM embodied in the aggregates (Šimanský, 2013). A low SOM content can lead to disturbance of aggregation and soil structure (Bernardes et al., 2004; Six et al., 2004). The use of imbalanced farming practices due to the intensification of agriculture poses a serious problem deteriorating soil fertility. The lack of organic fertilizers and removal of plant residues leads to a decrease in SOM content in soils. Regarding this issue, using biochar as a soil ameliorant could be a suitable alternative. Biochar is considered to be an important source of especially stable C compounds and its application to soil has the potential to increase the content of SOM in the long run (Gupta and Germida, 2015; Igaz et al., 2018; Kopittke et al., 2019; Šimanský et al., 2018b). Biochar is a solid, carbon-rich product and is formed during the thermochemical decomposition of various types of organic materials (Shackley et al., 2016). Application of biochar is reported to improve soil chemical (Juriga and Šimanský, 2019; Liang et al. 2006), physical (Balashov et al., 2019; Igaz et al., 2018; Toková et al., 2019; Vítková et al., 2017) and biological properties (Lehmann et al., 2011). Biochar also can increase crop yields (Kondrlová et al., 2017), reduce GHG emissions and increase soil carbon sequestration (Lehmann et al., 2006; Šimanský et al., 2018b). The use of biochar can offer many benefits to agriculture by improving the whole complex of soil properties, including soil structure.

However, the diverse range of effects of biochar on soil and crop parameters depends on its physicochemical properties. The latter are influenced by the conditions of biochar production (temperature and heating duration) and the origin of feedstock (Enders et al., 2012). At the same time, the soil-climatic conditions significantly affect the behavior of biochar after soil amendment. It was observed that the effect of biochar in sandy soils was more evident than in loamy or heavy clay soils (Blanco-Canqui, 2017). Biochar behavior in soil environment also depends on whether biochar is combined with fertilizers, as their interaction is also significant for soil structure parameters. N fertilization is necessary to reduce the wide C:N ratio that can significantly affect soil processes after biochar application (Spokas et al., 2015). In addition, there is still a lack of information in scientific literature on the effect of biochar reapplication on the soil properties, including soil structure.

Based on the above facts, we assumed that: (1) application of biochar will improve soil structure, (2) soil structure improvement will be more evident after application of a higher dose of biochar in comparison to a lower dose, (3) more stable soil structure will be observed in the case of biochar reapplication than in the case of biochar initial application, (4) N-fertilization will intensify the effect of biochar on soil structure and (5) the improvement of the soil structure parameters will be significantly related to an increase of the SOM content. The aim of this study was: (1) to evaluate the effects of initial biochar application and reapplication at different doses (0, 10 and 20 t $ha^{-1}$) alone and in combination with N fertilizer (40 to 240 kg N $ha^{-1}$ depending on the requirement of the cultivated crop) on the content of water-stable aggregates, (2) to assess the interrelationships between contents of SOM, humus and water-stable aggregates.

## MATERIALS AND METHODS

### Site characteristics

The study was conducted on the experimental site of the Slovak University of Agriculture in Nitra, located approximately 5 km from Nitra, Slovakia (Dolná Malanta, 48°19'00" N, 18°09'00" E). The experimental site is located in the western part of the Žitavská Pahorkatina hills, in the catchment of Selenec Stream, at an altitude of 176 m above sea level. The terrain is flat with a slight south-western gradient. The soil type is classified as Haplic Luvisol (IUSS, WRB, 2015) with silt loamy texture (Šimanský et al., 2018a). Soil pH prior to the experiment set up (in 2014) was 5.71 while the average soil organic carbon content was 9.31 g $kg^{-1}$ soil. Other soil properties are given in Table 1. The area belongs to warm temperate climate zone, fully humid with warm summers. The mean annual precipitation amount is 539 mm and the mean annual air temperature is 9.8 °C (Čimo et al., 2012).

**Table 1.** Soil properties prior to experiment establishment.

| Soil properties | Values |
|---|---|
| Clay (g $kg^{-1}$) | 249 |
| Silt (g $kg^{-1}$) | 599 |
| Sand (g $kg^{-1}$) | 152 |
| Soil organic carbon (g $kg^{-1}$) | 9.13 |
| CEC (mmol $kg^{-1}$) | 142 |
| Base saturation (%) | 85 |
| pH (in 1 mol.$dm^{-3}$ KCl) | 5.71 |

### Experiment description

The experiment with biochar in Dolná Malanta has been established in spring of 2014 before crop sowing. The crop rotation on the site was as follows: 2014 – spring barley (*Hordeum vulgare* L.), 2015 – maize (*Zea mays* L.), 2016 – spring wheat (*Triticum aestivum* L.), 2017 – maize, 2018 – spring barley and 2019 – maize. The experiment originally consisted of nine treatments (plot setting from 2014 up to 2017). The number of treatments increased by another six when biochar was applied for the second time (at the same rates as in 2014) in spring 2018 (plot setting from 2018 up to 2019). In total, the experiment currently consists of the following fifteen treatments:

1. N0B0–without N fertilizer and biochar application,
2. N0B10A–without N fertilizer and with 10 t $ha^{-1}$ of biochar,
3. N0B20A–without N fertilizer and with 20 t $ha^{-1}$ of biochar,
4. N0B10B–without N fertilizer and with 10 t $ha^{-1}$ of reapplied biochar,
5. N0B20B–without N fertilizer and with 20 t $ha^{-1}$ of reapplied biochar,
6. N1B0–with the first level of N fertilizer and without biochar application,
7. N1B10A–with the first level of N fertilizer and 10 t $ha^{-1}$ of biochar,
8. N1B20A–with the first level of N fertilizer and 20 t $ha^{-1}$ of biochar,
9. N1B10B–with the first level of N fertilizer and 10 t $ha^{-1}$ of reapplied biochar,
10. N1B20B–with the first level of N fertilizer and 20 t $ha^{-1}$ of reapplied biochar,
11. N2B0–with the second level of N fertilizer and without biochar application,
12. N2B10A–with the second level of N fertilizer and 10 t $ha^{-1}$ of biochar,
13. N2B20A–with the second level of N fertilizer and 20 t

ha$^{-1}$ of biochar,
14. N2B10B–with the second level of N fertilizer and 10 t ha$^{-1}$ of reapplied biochar,
15. N2B20B–with the second level of N fertilizer and 20 t ha$^{-1}$ of reapplied biochar.

The application doses of N fertilizer varied from year to year depending on the cultivated crop. The first level of N fertilization covered the usual N requirements of the cultivated crop to obtain the average yield. The second level of fertilization was 50% higher for maize and spring wheat (years 2015, 2017 and 2019) and 100% higher for spring barley (years 2014, 2016, 2018). Nitrogen fertilizer application doses (calculated as pure N) in the years evaluated in this study were 160 and 240 kg N ha$^{-1}$ (2017), 40 and 80 kg N ha$^{-1}$ (2018), and 160 and 240 kg N ha$^{-1}$ (2019) at the first and second fertilization level, respectively. During this period the same type of granulated N fertilizer – LAD27 (Duslo Šaľa a.s., Šaľa, Slovakia) was used. The fertilizer contained 27% of total N (equal amounts in ammoniacal and nitrate form) and 4.1% of magnesium oxide. The biochar (Sonnenerde, Riedlingsdorf, Austria) was produced from paper fiber sludge and grain husks (1:1). The feedstock was processed by pyrolysis at the temperature of 550 °C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). The composition and properties of biochar are shown in Table 2.

**Table 2.** Basic composition and properties of applied biochar.

| | |
|---|---|
| Ash (%) | 38.3 |
| Total C (%) | 53.1 |
| Total N (%) | 1.4 |
| Ca (g kg$^{-1}$) | 57 |
| Mg (g kg$^{-1}$) | 3.9 |
| K (g kg$^{-1}$) | 15 |
| Na (g kg$^{-1}$) | 0.7 |
| Specific surface area (m$^{-2}$ g$^{-1}$) | 21.7 |
| pH | 8.8 |
| Particle size range (mm) | 1–5 |

**Soil sampling**

The soil samples were collected in all treatments from a depth of 0–0.30 m. Soil sampling was carried out at monthly intervals during the growing season of maize, spring barley and maize in 2017, 2018 and 2019, respectively. In other words, the sampling in 2017 took place 38–43 months after initial biochar application in the spring of 2014 (9 average soil samples monthly, 54 soil samples in total for 2017). In 2018, it was already 49–53 months after initial biochar application (9 average soil samples monthly, 45 soil samples in total for 2018), but only 1–4 months after biochar reapplication in the spring of 2018 (6 another average soil samples monthly, 24 soil samples in total for 2018). In 2019, soil samples were collected 61–66 months after initial biochar application (9 another average soil samples monthly, 54 soil samples in total for 2019) and 12–16 months after biochar reapplication (6 another average soil samples monthly, 30 soil samples in total for 2019). A total of 207 soil samples were collected and subsequently analyzed for the period 2017–2019.

**Soil analytical methods**

The following parameters were determined in the air-dried soil samples: content of the individual size fractions of water-stable macro-aggregates ($WSA_{ma}$) (size fractions of > 5 mm, 5–3 mm, 3–2 mm, 2–1 mm, 1–0.5 mm, 0.5–0.25 mm and < 0.25 mm) by the Bakshayev method (Hrivňáková et al., 2011). Soil organic carbon content ($C_{org}$) was determined oxidometrically (Dziadowiec and Gonet, 1999). Labile carbon ($C_L$) content was determined using 0.005 mol dm$^{-3}$ $KMnO_4$ (Loginow et al., 1987). Composition of humus fractions (humic acids – HA and fulvic acids – FA) was determined according to the Belchikova and Kononova procedure (Dziadowiec and Gonet, 1999) and the absorbance of humic substances and humic acids was measured at 465 and 650 nm to calculate the color quotient $Q^{4/6}_{HS}$ and $Q^{4/6}_{HA}$ (Dziadowiec and Gonet, 1999).

**Statistical analysis**

The content of water-stable aggregates was evaluated by the statistical analysis in Statgraphics Centurion XV. I software (Statpoint Technologies, Inc., USA) using one-way analysis of variance (ANOVA). LSD test with a significance level of $\alpha = 0.05$ was used to compare the effects of the individual doses of initial and reapplied biochar and biochar combined with N fertilizer. Dependencies between the water-stable aggregates and the soil organic matter were evaluated using a simple correlation matrix and were expressed by a Pearson's correlation coefficient at the different probability levels.

## RESULTS

**Effects of the initial application of biochar and its combination with N fertilizer on water-stable aggregates**

The contents of water-stable macro-aggregates ($WSA_{ma}$) and water-stable micro-aggregates ($WSA_{mi}$) in investigated treatments for the period of 3 years are summarized in Figure 1.

Due to application of biochar alone as well as biochar in combination with both levels of N fertilizer, the total content of $WSA_{ma}$ has increased, while the content of $WSA_{mi}$ has decreased. The average results obtained 3–5 years after initial application of biochar to the soil confirmed the ameliorant's favorable effect on the soil structure. The changes were significant in the treatments where biochar was applied without any N-fertilizer. The content of $WSA_{ma}$ was significantly higher (+9% and +14% in relation to total content of $WSA_{ma}$) and the content of $WSA_{mi}$ significantly lower (–23% and –38% in relation to total content of $WSA_{mi}$) in the N0B10A and N0B20A treatments, respectively, when compared to the control treatment (N0B0). Thus, the effect of single biochar application at the rate of 20 t ha$^{-1}$ was more pronounced than at the rate of 10 t ha$^{-1}$. The effect of combined application of biochar with the first level N fertilizer was not significant. On the other hand, the significant changes were observed with application of 20 t ha$^{-1}$ of biochar in combination with the second level of N fertilizer (N2B20A): $WSA_{ma}$ content increased by 12% in relation to total content of $WSA_{ma}$ while $WSA_{mi}$ content decreased by 36% in relation to total content of $WSA_{mi}$ compared to the control at the second fertilization level (N2B0).

An increase in the content of the $WSA_{ma}$ larger size fraction (> 1 mm) was observed 3–5 years after the initial application of biochar (Table 3). In the case of the non-fertilized treatments with biochar, significantly higher contents of $WSA_{ma}$ (size fractions >5 mm, 5–3 mm, 3–2 mm and 2–1 mm) were observed in both treatments with biochar (N0B10A and N0B20A). The contents of these size fractions were respectively higher by 50%, 46%, 36% and 39% for N0B10A treatment and by 63%, 63%, 46% and 39% for N0B20A treatment in comparison to the control N0B0. At the same time, a significantly higher content of agronomically valuable size fraction of $WSA_{ma}$ (3–0.5 mm) was also found. In the case of N0B10A and N0B20A treatments, the content of this size fraction of $WSA_{ma}$

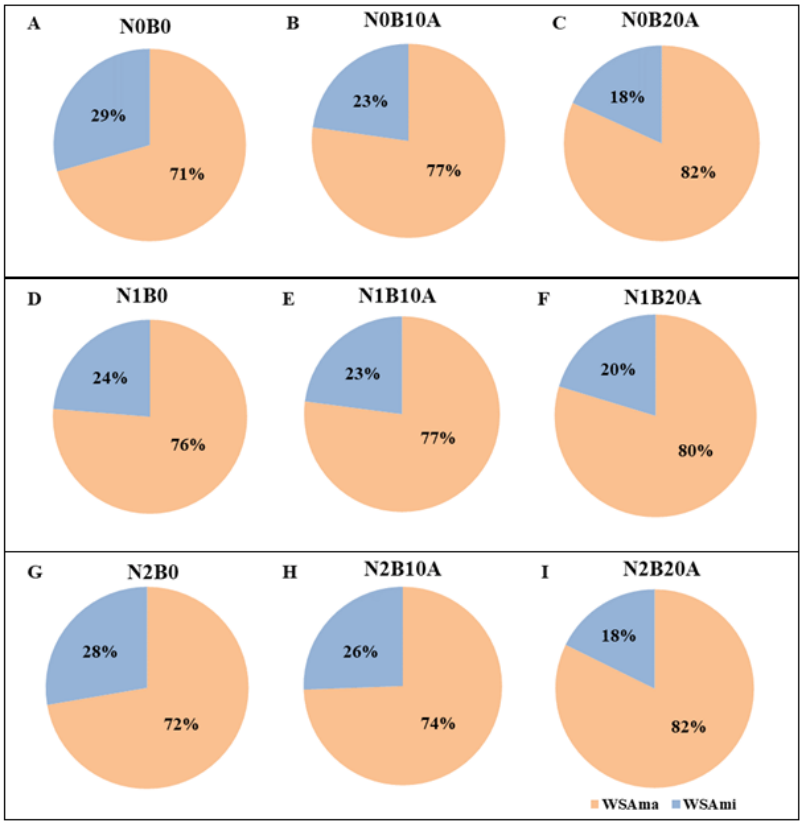



**Fig. 1.** Content (%) of water-stable macro-aggregates and micro-aggregates after the initial application of biochar (in 2014) treatments: (A) control (without biochar and N fertilizer application), (B) biochar at a rate of 10 t ha$^{-1}$, (C) biochar at a rate of 20 t ha t ha$^{-1}$, (D) control (at the first level of N fertilization), (E) biochar at a rate of 10 t ha$^{-1}$ + first level of N, (F) biochar at a rate of 20 t ha$^{-1}$ + first level of N, (G) control (at the second level of N fertilization), (H) biochar at a rate of 10 t ha$^{-1}$ + second level of N, (I) biochar at a rate of 20 t ha$^{-1}$ + second level of N.

**Table 3.** Percentage representation of individual size fractions of water-stable macro-aggregates after the initial application of biochar (in 2014).

| **$WSA_{ma}$ content (%)** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **> 5 mm** | **5–3 mm** | **3–2 mm** | **2–1 mm** | **1–0.5 mm** | **0.5–0.25 mm** | **0.5–3 mm** |
| **N0B0** | 1.41±0.13$^{a}$ | 2.57±0.07$^{a}$ | 6.49±1.07$^{a}$ | 10.2±2.66$^{a}$ | 29.4±5.86$^{a}$ | 20.5±5.25$^{a}$ | 46.1±4.06$^{a}$ |
| **N0B10A** | 2.81±0.57$^{b}$ | 4.75±1.05$^{b}$ | 10.2±0.76$^{b}$ | 16.7±2.79$^{b}$ | 25.9±5.83$^{a}$ | 16.9±3.77$^{a}$ | 52.7±1.58$^{b}$ |
| **N0B20A** | 3.82±0.60$^{b}$ | 6.89±0.77$^{c}$ | 12.0±1.22$^{b}$ | 16.7±1.91$^{b}$ | 25.9±6.28$^{a}$ | 16.4±7.04$^{a}$ | 54.6±1.16$^{b}$ |
| **N1B0** | 2.73±1.50$^{a}$ | 4.14±2.82$^{a}$ | 8.61±3.96$^{a}$ | 14.9±8.66$^{a}$ | 28.6±4.63$^{a}$ | 17.3±5.72$^{a}$ | 52.1±11.56$^{a}$ |
| **N1B10A** | 3.15±2.33$^{a}$ | 4.86±2.46$^{a}$ | 9.25±3.53$^{a}$ | 12.6±6.25$^{a}$ | 30.4±7.53$^{a}$ | 16.8±4.24$^{a}$ | 52.2±8.07$^{a}$ |
| **N1B20A** | 2.24±1.20$^{a}$ | 4.36±2.66$^{a}$ | 10.9±6.43$^{a}$ | 17.4±8.84$^{a}$ | 28.3±6.54$^{a}$ | 16.6±5.61$^{a}$ | 56.6±11.38$^{a}$ |
| **N2B0** | 2.09±0.48$^{a}$ | 2.76±0.89$^{a}$ | 6.57±2.03$^{a}$ | 12.4±3.16$^{a}$ | 28.5±4.34$^{a}$ | 19.9±2.41$^{b}$ | 47.5±3.10$^{a}$ |
| **N2B10A** | 1.83±0.38$^{a}$ | 3.90±0.65$^{ab}$ | 7.62±1.09$^{a}$ | 13.3±2.76$^{ab}$ | 29.9±6.79$^{a}$ | 17.8±0.91$^{ab}$ | 50.9±3.22$^{a}$ |
| **N2B20A** | 3.48±0.54$^{b}$ | 5.70±1.16$^{b}$ | 11.9±2.70$^{b}$ | 19.4±2.86$^{b}$ | 27.7±7.85$^{a}$ | 14.1±3.41$^{a}$ | 59.0±2.77$^{b}$ |

The description of the treatment acronyms is given in the Materials and Methods Section. The different letters (a, b, c) between the rows express statistical significance according to the LSD test. $WSA_{ma}$ – water-stable macro-aggregates.

was, respectively, 13% and 16% higher compared to the control N0B0 (Table 3). A significant difference in $WSA_{ma}$ content was also observed between the two biochar treatments: the content of the aggregate size fraction 5–3 mm in N0B20A treatment was significantly higher (+31%) in comparison to N0B10A treatment. The initial biochar application in combination with the first level of N fertilizer did not significantly affect the content of any $WSA_{ma}$ size fractions. However, at the second N fertilization level, significant changes in the content of almost all the $WSA_{ma}$ size fractions (except the fraction of 1–0.5 mm) were observed at the higher rate of biochar application. In this case, the trend in $WSA_{ma}$ content was similar to the biochar higher rate application without fertilizer. For the N2B20A treatment, a significantly higher proportion of $WSA_{ma}$ was

determined for size fractions of > 5 mm, 5–3 mm, 3–2 mm and 2–1 mm (+40%, +52%, +45% and +36%, respectively), when compared to the control at the second N fertilization level. At the same time, a proportion of $WSA_{ma}$ of 0.5–0.25 mm was 29% lower. The content of agronomically valuable aggregate size fraction (3–0.5 mm) in the N2B20A treatment was also higher (by 20%) than in the N control. In addition, a significantly higher $WSA_{ma}$ content was observed in N2B20A treatment for size fractions > 5 mm (+ 47%), 3–2 mm (+ 36%) and 3–0.5 mm (+ 14%) when compared to N2B10A treatment.

**Effect of biochar and biochar in combination with N fertilizer reapplication on the contents of water-stable aggregates**

Soil structure has also significantly improved after reapplication of biochar or biochar in combination with N fertilizer (Fig. 2). After reapplication of biochar alone, a significant increase in a $WSA_{ma}$ content was observed in the two treatments with biochar (9% for N0B10B treatment and 15% for N0B20B treatment – in relation to total content of $WSA_{ma}$) when compared to the control. As the $WSA_{ma}$ content increased, the $WSA_{mi}$ content decreased significantly: by 24% in N0B10B treatment and by 41% in N0B20B treatment in relation to total content of $WSA_{mi}$. Similar to the initial application of biochar, reapplication of biochar alone was more favorable at a rate of 20 t $ha^{-1}$ rather than of 10 t $ha^{-1}$. The obtained results confirm no significant effect from reapplication of biochar in combination with the first level of N fertilizer. However, when biochar was reapplied together with the second level of N fertilizer, higher $WSA_{ma}$ contents (+12% and +11% in relation to total content of $WSA_{ma}$) and lower $WSA_{mi}$ contents (–35% and –30% in relation to total content of $WSA_{mi}$) were found in N2B10B and N2B20B treatments, respectively.

The mean values observed during the first two years (2018–2019) after biochar reapplication also showed positive changes in the content of individual $WSA_{ma}$ size fractions (Table 4). For this parameter, a higher efficiency of biochar reapplication at a rate of 20 t $ha^{-1}$ rather than 10 t $ha^{-1}$ was also confirmed. After reapplication of biochar at a lower rate (N0B10B), the $WSA_{ma}$ content of the three largest size fractions of > 5 mm, 5–3 mm and 3–2 mm increased significantly by 60%, 60% and 41%, respectively. Biochar reapplication at the higher rate (N0B20B) resulted in a significant increase of the $WSA_{ma}$ content in the four largest size fractions of >5 mm, 5–3 mm, 3–2 mm and 2–1 mm by 72%, 65%, 57% and 64%, respectively. At the same time the content of $WSA_{ma}$ of the size fraction 0.5–0.25 mm decreased by 25% when compared to the control. Moreover, in the case of N0B20B treatment, a significantly higher content (+17%) of agronomically valuable $WSA_{ma}$ size fraction (3–0.5 mm) was also observed when compared to the control. In addition, the significant differences were also observed between the two biochar only treatments. The N0B20B treatment showed a significantly higher content of $WSA_{ma}$ in 5–3 mm, 3–2 mm and 2–1 mm size fractions (+13%, +28% and +28%, respectively) when compared to the N0B10B treatment. No significant effect of biochar reapplication in combination with the first level of N fertilizer on the content of any $WSA_{ma}$ size fractions was found. In contrast, biochar reapplication in combination with the second level of N fertilizer, resulted in a significantly higher $WSA_{ma}$ content of 5–3 mm size fraction (+45% for N2B10B treatment) and 3–2 mm size fraction (+44% for N2B20B treatment) compared to the N-control. At the same time, the content of $WSA_{ma}$ size fraction 0.5–0.25 mm significantly decreased (–26% for N2B20B treatment). In addition, the content of the agronomically valuable $WSA_{ma}$ size fraction (3–0.5 mm) increased significantly in both treatments: 19% and 22% for N2B10B and + N2B20B treatments, respectively.

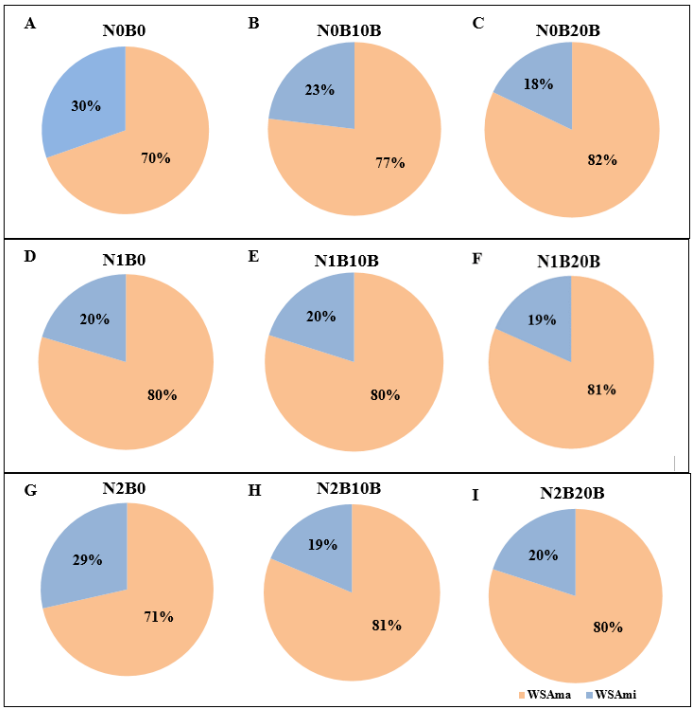



**Fig. 2.** Content (%) of water-stable macro-aggregates and micro-aggregates in the treatments with reapplied biochar (initial 2014 and reapplied in 2018) in individual treatments: (A) control (without biochar and N fertilizer application), (B) reapplied biochar at a rate of 10 t $ha^{-1}$, (C) reapplied biochar at a rate of 20 t ha t $ha^{-1}$, (D) control (at the first level of N fertilization), (E) reapplied biochar at a rate of 10 t $ha^{-1}$ + first level of N, (F) reapplied biochar at a rate of 20 t $ha^{-1}$ + first level of N, (G) control (at the second level of N fertilization), (H) reapplied biochar at a rate of 10 t $ha^{-1}$ + second level of N, (I) reapplied biochar at a rate of 20 t $ha^{-1}$ + second level of N.

**Table 4.** Percentage representation of individual size fractions of water-stable macro-aggregates in the treatments with reapplied biochar (in 2018).

| $WSA_{ma}$ content (%) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **> 5 mm** | **5–3 mm** | **3–2 mm** | **2–1 mm** | **1–0.5 mm** | **0.5–0.25 mm** | **0.5–3 mm** |
| **N0B0** | 1.64±1.10$^{a}$ | 2.37±0.70$^{a}$ | 5.66±1.87$^{a}$ | 8.79±1.43$^{a}$ | 29.3±6.75$^{a}$ | 21.9±2.65$^{b}$ | 43.7±5.29$^{a}$ |
| **N0B10B** | 4.07±1.87$^{b}$ | 5.97±0.66$^{b}$ | 9.55±1.32$^{b}$ | 9.90±2.73$^{a}$ | 29.8±5.18$^{a}$ | 17.7±3.78$^{ab}$ | 49.2±4.49$^{ab}$ |
| **N0B20B** | 5.80±1.68$^{b}$ | 6.86±0.85$^{c}$ | 13.3±1.38$^{c}$ | 13.8±1.46$^{b}$ | 25.8±3.49$^{a}$ | 16.5±2.04$^{a}$ | 52.9±5.48$^{b}$ |
| **N1B0** | 3.24±1.62$^{a}$ | 4.55±1.26$^{a}$ | 8.45±2.95$^{a}$ | 16.5±4.02$^{a}$ | 28.8±2.61$^{a}$ | 18.1±5.92$^{a}$ | 53.7±10.6$^{a}$ |
| **N1B10B** | 3.22±1.67$^{a}$ | 4.88±2.30$^{a}$ | 8.90±2.85$^{a}$ | 15.2±7.98$^{a}$ | 29.5±2.52$^{a}$ | 18.2±3.79$^{a}$ | 53.6±9.05$^{a}$ |
| **N1B20B** | 2.81±1.19$^{a}$ | 3.57±0.92$^{a}$ | 17.4±1.48$^{a}$ | 14.2±6.17$^{a}$ | 35.9±1.07$^{b}$ | 17.5±3.04$^{a}$ | 57.5±6.34$^{a}$ |
| **N2B0** | 2.47±1.67$^{a}$ | 2.47±0.69$^{a}$ | 5.38±1.30$^{a}$ | 10.5±7.11$^{a}$ | 29.6±3.67$^{a}$ | 21.1±1.87$^{b}$ | 45.5±5.81$^{a}$ |
| **N2B10B** | 2.73±1.16$^{a}$ | 4.48±0.68$^{b}$ | 9.07±2.10$^{ab}$ | 15.9±8.30$^{a}$ | 31.5±8.16$^{a}$ | 17.7±3.72$^{ab}$ | 56.5±7.38$^{b}$ |
| **N2B20B** | 2.20±1.04$^{a}$ | 3.63±0.70$^{ab}$ | 9.67±1.76$^{b}$ | 15.4±8.72$^{a}$ | 33.5±5.38$^{a}$ | 15.6±3.08$^{a}$ | 58.6±8.62$^{b}$ |

The description of the treatment acronyms is given in the Materials and Methods Section. The different letters (a, b, c) between the rows express statistical significance according to the LSD test. $WSA_{ma}$ – water-stable macro-aggregates.

**Table 5.** Correlation relationships between parameters of soil organic matter, humus and soil structure parameters for the treatments with initial biochar application (in 2014).

| **Parameter** | **$WSA_{ma}$** | **$WSA_{mi}$** | **Individual size fractions of $WSA_{ma}$ (mm)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **> 5** | **5–3** | **3–2** | **2–1** | **1–0.5** | **0.5–0.25** | **0.5–3** |
| **$C_{org}$** | 0.268* | –0.268* | 0.436*** | 0.277* | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$C_L$** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$C_{HS}$** | –0.329** | 0.329** | –0.346** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$C_{HA}$** | –0.342** | 0.342** | –0.375** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$C_{FA}$** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$C_{HA}$:$C_{FA}$** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| **$Q_{HS}$** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | –0.262* | n. s. |
| **$Q_{HA}$** | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | –0.271* | n. s. |

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, n. s. – nonsignificant.
$C_{org}$–total organic carbon, $C_L$–labile carbon, $C_{HS}$–carbon of humic substances, $C_{HA}$–carbon of humic acids, $C_{FA}$–carbon of fulvic acids, $C_{HA}$:$C_{FA}$–ratio between carbon of humic acids and carbon of fulvic acids, $Q_{HS}$–color quotient of humic substances, $Q_{HA}$–color quotient of humic acids, $WSA_{ma}$–water-stable macro-aggregates, $WSA_{mi}$–water-stable micro-aggregates.

### Evaluation of relationships between parameters of soil organic matter and water-stable aggregates affected by initial biochar application

Correlations between contents of soil organic matter (SOM) and water-stable aggregates depending on the initial biochar application are shown in Table 5. The results of our study showed that for the period 2017–2019 the soil organic carbon ($C_{org}$) content had a positive effect on the content of $WSA_{ma}$. At the same time, the content of $C_{org}$ significantly affected the content of the two largest $WSA_{ma}$ size fractions (> 5 mm and 5–3 mm) but did not affect the content of the smaller water-stable aggregates.

No significant correlations were observed between soil structure and the soil labile carbon content ($C_L$). The obtained results also showed that the extractable carbon of humic substances ($C_{HS}$) and humic acids ($C_{HA}$) had a significant negative correlation with $WSA_{ma}$ content as well as with the content of the largest $WSA_{ma}$ size fraction (> 5 mm). In addition, the color quotients of humic substances ($Q_{HS}$) and humic acids ($Q_{HA}$), which are the expression of the humic substances and humic acids stability, were in negative correlation with the content of the smallest $WSA_{ma}$ size fraction (0.5–0.25 mm).

### Evaluation of relationships between parameters of soil organic matter and water-stable aggregates affected by biochar reapplication

According to our results, the $C_{org}$ significantly contributed to a higher content of $WSA_{ma}$ and to a content of agronomically valuable $WSA_{ma}$ size fraction after biochar reapplication into the soil (Table 6). A significant effect of $C_{org}$ on the content of the three largest $WSA_{ma}$ size fractions (> 5 mm, 5–3 mm and 3–2 mm) was also demonstrated, while it had a significant negative effect on the content of the smallest $WSA_{ma}$ size fraction. $C_L$ content after biochar reapplication significantly contributed to the formation of $WSA_{ma}$ of 1–0.5 mm. $C_{HS}$ and $C_{HA}$ contents negatively correlated with the content of $WSA_{ma}$ size

**Table 6.** Correlation relationships between parameters of soil organic matter, humus and soil structure parameters in the treatments with reapplied biochar (initial 2014 and reapplied in 2018).

| Parameter | $WSA_{ma}$ | $WSA_{mi}$ | Individual size fractions of $WSA_{ma}$ (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | > 5 | 5–3 | 3–2 | 2–1 | 1–0.5 | 0.5–0.25 | 0.5–3 |
| $C_{org}$ | 0.342** | –0.342** | 0.326** | 0.313* | 0.447*** | n. s. | n. s. | –0.303* | 0.287* |
| $C_L$ | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | 0.262* | n. s. | n. s. |
| $C_{HS}$ | –0.299* | 0.299* | –0.328** | –0.326** | –0.380** | n. s. | n. s. | 0.265* | n. s. |
| $C_{HA}$ | –0.330** | 0.330** | –0.319* | –0.359** | –0.453*** | n. s. | n. s. | 0.268* | n. s. |
| $C_{FA}$ | n. s. | n. s. | –0.276* | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| $C_{HA}$:$C_{FA}$ | n. s. | n. s. | n. s. | n. s. | –0.328** | n. s. | n. s. | n. s. | n. s. |
| $Q_{HS}$ | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |
| $Q_{HA}$ | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. | n. s. |

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, n. s. – nonsignificant.
$C_{org}$–total organic carbon, $C_L$–labile carbon, $C_{HS}$–carbon of humic substances, $C_{HA}$–carbon of humic acids, $C_{FA}$–carbon of fulvic acids, $C_{HA}$:$C_{FA}$–ration between carbon of humic acids to carbon of fulvic acids, $Q_{HS}$–color quotient of humic substances, $Q_{HA}$–color quotient of humic acids, $WSA_{ma}$–water-stable macro-aggregates, $WSA_{mi}$–water-stable micro-aggregates.

fractions of > 5 mm, 5–3 mm and 3–2 mm and positively correlated with the content of 0.5–0.25 mm size fraction of $WSA_{ma}$. In addition, the $C_{HA}$:$C_{FA}$ ratio negatively correlated with the content of 3–2 mm size fraction of $WSA_{ma}$.

## DISCUSSION

### Effects of initial application of biochar and biochar with N-fertilizer on soil structure

Up to date, several authors have described an improvement in soil structural status due to application of biochar (Gupta and Germida, 2015; Juriga et al., 2018; Lu et al., 2014; Obia et al., 2016; Sing and Cowie, 2014; Šimanský et al., 2016). For soil structure evaluation, $WSA_{ma}$ content is one of the most suitable and popular parameters as it expresses the representation of individual size fractions of macro-aggregates and their resistance to water forces (Scott, 2000). According to Šimanský and Bajčan (2014), content of $WSA_{ma}$ size fraction of 3–0.5 mm is the most valuable for agronomic practice, because at the optimal content of these aggregates in a soil, favorable physical conditions, suitable for most of the cultivated crops, are being formed. The content of these aggregates was significantly higher in N0B10A (+13%) and N0B20A (+16%) treatments when compared to the control. In overall, biochar application at a rate of 20 t $ha^{-1}$ proved to be more effective than at a rate of 10 t $ha^{-1}$. This finding corresponds to the statement of Blanco-Canqui (2017), according to which the amount of applied biochar is a key factor in the extent of its effects on soil structure. In our case, rates of 10 t $ha^{-1}$ and 20 t $ha^{-1}$ of biochar were used, however from the available literature it is clear that much higher rates are also often used in other experiments. For example, Dong et al. (2016) tested the efficiency of biochar application at rates of 30 t $ha^{-1}$, 60 t $ha^{-1}$ and 90 t $ha^{-1}$. The results of their three-year study showed that the content of $WSA_{ma}$ increased significantly when compared to the control only in case of biochar application at a rate of 60 t $ha^{-1}$ (+45%). Their study was performed on a sandy soil and, as pointed out by Blanco-Canqui (2017), the effect of biochar on soil structure improvement to a large extent also depends on soil texture. Although more pronounced effects are usually observed on sandy soils, as reported by Gul et al. (2015), the properties of biochar, depending on the pyrolysis temperature and the type of feedstock, are crucial. Thus, biochar made from the material rich in cellulose and at a high temperature usually has a lower content of nutrients and aliphatic C compounds. In addition, it has a larger specific surface area and sorption capacity (Igaz et al., 2018). These properties of biochar can have a negative effect on aggregation, especially in sandy soils, which are poor in SOM and nutrients, and where the microbial activity leading to aggregation can be suppressed. On the other hand, Demisie et al. (2014) observed an increased stability of soil aggregates due to an increase of SOM and microbial activity after biochar (made of wood at a temperature of 600 °C) application into the clay soil. In our case, biochar was made from cereal husks and paper fiber sludge in a ratio of 1:1 and at a pyrolysis temperature of 550 °C. Biochars are generally characterized by a broad C:N ratio, which may contribute to the lack of N for plants as well as soil microorganisms after its depletion from the soil. Therefore, it appears important to apply N-rich fertilizers, which contribute to a narrower C:N ratio and promote plant growth and microbial activity, together with biochar (Spokas et al., 2015). For this reason, we also included into the experiment the treatments where biochar application was combined with mineral N fertilization at two different application levels. Mineral fertilizers are an important source of nutrients for plants as well as for soil microorganisms. Through their application, the production of root and above-ground plant biomass can be increased, which contributes to a higher SOM content and soil structure improvement. However, an increase in microbial activity may increase the degree of SOM mineralization, which in turn may worsen the soil structure conditions (Dong et al., 2014; Geisseler and Scow, 2014). Since biochar is a source of mainly stable aromatic C compounds, it can reduce the decomposition of organic C by stabilizing its labile fractions and thus promote aggregation (Elzobair et al., 2017; Jien et al., 2015). These statements are in agreement with our findings, as significantly better soil structure was formed in the case of biochar application at the rate of 20 t $ha^{-1}$ combined with the second level of N fertilizer when compared to N fertilization alone.

### Effects of reapplication of biochar and biochar with N fertilizer on water-stable aggregates

Biochar applied to the soil undergoes gradual changes, which also cause a change in its effects on the soil properties. Some of the biochar effects on soils and crops can be weakened

or even lost during the process of biochar aging. For this reason, it is rational to consider repeated application of biochar to soils at certain time intervals (Blanco-Canqui, 2017; Ren et al., 2018). Regarding soil structure, different effects can be observed shortly after the application of biochar. Due to its predominantly aromatic structure, biochars are characterized by a relatively low content of aliphatic C compounds, which are released into the soil shortly after the incorporation of biochar and are considered an important element for the formation and stability of soil aggregates (Moreno-Barriga et al., 2017; Mukherjee and Lal, 2013). On the other hand, as Blanco-Canqui, (2017) claimed, the effects on the soil structure change become apparent only after a certain time after biochar application. In our case, biochar reapplication significantly improved the soil structure parameters already during the first two years. This is proven by the significantly higher $WSA_{ma}$ content in both biochar alone treatments: N0B10B and N0B20B (+9% and +15%, respectively) as well as by the improvement of other evaluated soil structure parameters when compared to the control. The use of mineral N fertilizers often leads to a decrease in soil pH, which can result in worse soil structure (Haynes and Naidu, 1998). However, Bethlenfalvay et al. (1999) pointed out that N fertilization can significantly improve the structural conditions of soils (in alkaline soils) also through the acidifying effect. They found that lowering pH due to N fertilization promoted the activity of soil fungi, thereby increased the formation and stability of soil aggregates. On the other hand, Fungo et al. (2017) tested the effect of urea alone and urea in combination with biochar on soil structure. They observed that fertilization with urea alone did not have a significant effect on the $WSA_{ma}$ content, but in combination with biochar, 15% higher $WSA_{ma}$ content was found. A significant improvement of soil structure due to combined reapplication of biochar and N fertilizer when compared to N fertilizer alone was also observed in our study at the second level of N fertilization. In our case, LAD 27 was used as an N fertilizer and the fertilizer's physiological acidic effect was eliminated by added dolomite. Overall, alkaline biochar tends to increase soil pH, especially of acid soils, which was also our case (Horák, 2015; Šimanský et al., 2018a). At the same time, after biochar reapplication, we found a significant increase in the content of larger $WSA_{ma}$ (size fraction of > 5–1 mm). The beneficial effect of biochar reapplication on the individual $WSA_{ma}$ size fractions was also reported by Liu et al. (2014) for sandy soils. However, these authors observed a significant increase in the content of larger and smaller $WSA_{ma}$ size fractions of > 2 mm (+33%), 2–0.5 mm (+28%) and 0.5–0.25 mm (+24%) when compared to the control. According to Wang et al. (2018) long-term mineral N fertilization tends to increase the proportion of especially larger $WSA_{ma}$ size fractions (> 2 mm). For example, Wang et al. (2014) found that N fertilization significantly increased the proportion of macro-aggregates of larger size fractions (> 2 mm) by +7% when compared to the control. However, the share of smaller macro-aggregates did not significantly increase. The authors explained that this fertilizing effect was a result of an increase in root biomass and fungal mycelium in these aggregate size fractions, what is consistent with the statement of Bronick and Lal (2005) that plant roots and fungal mycelium are important factors influencing the formation of larger macro-aggregates in particular. Our results further showed that the effect of combined reapplication of 10 t $ha^{-1}$ and 20 t $ha^{-1}$ of biochar together with the second level of N fertilizer was significantly stronger than the effect of N fertilizer alone. In the N2B10B treatment a significantly higher $WSA_{ma}$ content of 5–3 mm size fraction was found (+45%) in comparison with the N-control and in the treatment N2B20B it was +44% for 3–2 mm size fraction of $WSA_{ma}$. However, at the first level of N fertilization, no significant difference was found between the treatments with a combination of biochar and N fertilizer and the treatment with N fertilizer alone.

**Relationship between soil organic matter and water-stable aggregates influenced by initial biochar application**

The effects of initial biochar application and biochar with N fertilizer on the parameters of SOM during the period of 2017–2019 have been partially published (Juriga et al., 2018). The formation of soil aggregates is generally dependent on the presence of cementing agents combining the soil particles into micro-aggregates and micro-aggregates into macro-aggregates (Bronick and Lal, 2005; Tisdall and Oades, 1982). As already mentioned, SOM is a key component in the soil aggregation process. For this reason, the addition of organic matter to the soil is considered to be an effective tool to improve soil structure, especially in soils with low SOM content (Bronick and Lal, 2005; Demisie et al., 2014). Biochar has the potential to increase $C_{org}$ content in soil in the long run (Gupta and Germida, 2015; Juriga et al., 2018; Lu et al., 2014), which corresponds to the findings of Juriga et al. (2018), who observed a significant increase in $C_{org}$ content due to initial application of biochar. The results of our study showed that the increased content of $C_{org}$ significantly contributed to the formation of larger size fractions of $WSA_{ma}$ (> 5 and 5–3 mm) and to the improvement of the parameters describing stability of water-stable macro-aggregates. $C_L$ content is an important indicator of soil quality, which can also significantly contribute to the formation and stability of soil aggregates (Šimanský, 2013), which has been proven so far in many studies (e. g. Juriga et al., 2018; Polláková et al., 2018; Šimanský et al., 2016). However, in our case, soil structure was not significantly affected by the $C_L$ content. Juriga et al. (2018) reported a statistically significant decrease in the extractability of $C_{HS}$, $C_{HA}$ and $C_{FA}$ due to initial application of biochar. The humic substances are also considered to be an important cementing agent involved in the formation and stability of soil aggregates (Bronick and Lal, 2005; Jindo et al., 2016; Spaccini et al., 2002). As reported by Mierzwa-Hersztek et al. (2018), biochar added to the soil could result in a decrease of the humic acid carbon and fulvic acid carbon in relation to the total organic carbon content, what in our case could be the reason of observed significant negative correlations between $C_{HS}$, $C_{HA}$ contents and $WSA_{ma}$ content after the initial application of biochar (Table 5).

**Relationships between soil organic matter and water-stable aggregates influenced by biochar reapplication**

In soils where SOM is the main substance affecting the soil structure, a role of $C_{org}$ in the formation of individual aggregate size fractions tends to increase with aggregate size (Lugato et al., 2010; Wang et al., 2017). This statement is in agreement with our findings, as the $C_{org}$ content contributed to the formation of the three largest $WSA_{ma}$ size fractions (> 5 mm, 5–3 mm and 3–2 mm). According to Demisie et al. (2014), biochar reapplication can significantly increase the $C_L$ content in the soil, but this effect tends to be short-term. In addition, biochar particles can bind $C_L$ from the soil, which can reduce its content in the soil and thus reduce its participation in aggregation. The $C_L$ content after biochar reapplication significantly contributed to the formation of $WSA_{ma}$ of 1–0.5 mm but did not increase the stability of water-stable macro-aggregates. Humic acids can

react with clay minerals to form organic-mineral colloids, which are the foundation for soil aggregates. At the same time, humic acids can be absorbed by metal oxides, changing their electric charge and thus increasing their participation in soil aggregation (Bronick and Lal, 2005). Due to its high surface activity, biochar can bind humic acids on its surface, which increases its reactivity and thus its contribution to the formation of soil structure (Liang et al., 2015). However, in our case we found significant negative correlations between soil structure and the humic substances after biochar reapplication (Table 6), which could be explained in a decrease of the humic acid carbon and fulvic acid carbon in relation to the total organic carbon content after biochar reapplication as it was published in this experiment by Juriga et al. (2018). Zhao et al. (2017) also pointed out that such results may be related to the properties of the biochar used. They found that biochar produced at lower pyrolysis temperatures (300 °C and 400 °C) had a higher content of labile C compounds, which were preferentially incorporated into humic substances contributing to an increase in $C_{HS}$ in the soil. On the other hand, biochar produced at higher pyrolysis temperatures (500 °C and 600 ° C) was more stable and caused a decrease in $C_{HS}$ in the soil, which could be explained by the microbial decomposition induced by the lack of labile C compounds in the soil. In our case, biochar was produced at a pyrolysis temperature of 550 °C. This fact could significantly affect the relationships between SOM and soil structure.

## CONCLUSIONS

The evaluation of the cumulative effects of biochar three up to five years after its initial application and one up to two years after its reapplication confirmed the significant soil structure improvement. Overall, more pronounced changes were observed after biochar reapplication than after its initial application. In both cases, most of the parameters of soil structure were more favorably affected by application of biochar alone rather than by its combination with N fertilizer. Application of biochar alone at a rate of 20 t $ha^{-1}$ was more effective than at a rate of 10 t $ha^{-1}$. The highest number of significant changes in soil structure was found in the treatment with reapplied biochar at a rate of 20 t $ha^{-1}$ without N fertilizer in comparison with the control. The soil structure formation was significantly supported by rather an increase in the soil organic matter content than its composition, both after the initial application and reapplication of biochar.

Due to the beneficial effects not only on soil structure but also on the whole range of other soil properties, biochar could find its practical application in agricultural soils as a soil ameliorant in the future. Considering the current situation of insufficient inputs of organic matter into agricultural soils, biochar could be a suitable partial substitute for its ability to improve some soil properties. Our findings confirmed the beneficial effects of biochar application on soil structure improvement in silt loamy soil. Therefore, it can be recommended now to focus on sandy and clay soils in the climatic conditions similar to Slovakia in future research. It is also important to observe the trends in the biochar effects on soil properties over the years after application to the soil, because, as our results suggest, the extent of biochar effect on soil structure may change over time. We believe that our results will contribute to a raising awareness and will fill knowledge gaps related to biochar use not only in Slovakia but also worldwide.

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## REFERENCES

Balashov, E., Mukhina, I., Rizhiya, E., 2019. Differences in water vapor adsorption-desorption of non aged and 3-year aged biochar in sandy Spodosols. Acta Hort. Regiotec., 22, 57–61.

Beck-Broichsitter, S., Ruth, S., Schröder, R., Fleige, H., Gerke, H. H., Horn, R., 2020. Simultaneous determination of wettability and shrinkage in an organic residue amended loamy topsoil. J. Hydrol. Hydromech., 68, 111–118.

Bernardes, M.C., Martinelli, L.A., Krusache, A.V., Gudeman, J., Moreira, M., Victoria, R.L., Ometto, P.H.B., Balleser, M.V.R., Autenkampe, K.A., Richey, J.E., Hedges, J.I., 2004. Riverine organic matter composition as a function of land use changes, southwest Amazon. Ecol. Soc. Am., 14, 263–279.

Bethlenfalvay, G.J., Cantrell, I.C., Mihara, K.L., Schréner, R.P., 1999. Relationships between soil aggregation and mycorhizae as influenced by soil biota and nitrogen nutrition. Biol. Fertil. Soils., 28, 356–363.

Blanco–Canqui, H., 2017. Biochar and soil physical properties. Soil Sci. Am. J., 81, 687–711.

Bogunovic, I., Telak, L.J., Pereira, P., Filipovic, V., Filipovic, L., Percin, A., Durdevic, B., Birkás, M., Dekemati, I., Comino, J.R., 2020. Land management impacts on soil properties and initial soil erosion processes in olives and vegetable crops. J. Hydrol. Hydromech., 68, 328–337.

Bronick, C.J., Lal, R., 2005. The soil structure and land management: a review. Geoderma, 124, 3–22.

Carter, M.R., Stewart, B.A., 1996. Structure and Organic Matter Storage in Agricultural Soils. CRC/Lewis Publisher, Boca Raton, 289 p.

Czachor, H., Lichner, Ľ., 2013. Temperature influences water sorptivity of soil aggregates. J. Hydrol. Hydromech. 61, 84–87.

Čimo, J., Špánik, F., Šiška, B., Tomlain, J., Horák, J., 2012. Practical Biometeorology. Slovak University of Agriculture, Nitra, Slovakia, 178 p. (In Slovak.)

Demisie, W., Liu, Z., Zhang, H., 2014. Effect of biochar on carbon fractions and enzyme activity of red soil. Catena, 121, 214–221.

Dong, W.Y., Zhang, X.Y., Dai, X.Q., Fux, L., Yang, F.T., Liu, X.Y., Sun, X.M., Wen, X.F., Schaeffer, S., 2014. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. Appl. Soil Eco., 84, 140–147.

Dong, X., Li, G., Guan, T., Lyn, Q., 2016. Long-term effect of biochar amount on the control and composition of organic matter in soil aggregates under field conditions. J. Soils Sediments, 16, 142–145.

Dziadowiec, H., Gonet, S.S., 1999. Estimation of fractional

composition of soil humus by Kononova–Bielcikova's method. Methodical guide-book for soil organic matter studies. Prace Komisji Naukowych Polskiego Towarzystwa Naukowego 120, Warszawa, Poland, pp. 31–34.

Elzobair, K., Alkanami, M.T., Alasvud, A., 2017. Effect of manure levels on growth and yield of yellow corn in sandy soil under dry climate. J. Pure Appl. Sci., 16, 147–150.

Enders, A., Manlei, K., Whitman, T., Joseph, S., Lehmann, J., 2012. Characterization of biochars to evaluate recaltritate and agronomic performance. Bioresource Technol., 114, 644–653.

Fiedler, H.J., Reissig, H., 1964. Lehrbuch der Bodenkunde (Textbook of Soil Science). VEB Fischer Verlag, Jena, 544 p. (In German.)

Fulajtár, E., 2006. Physical Properties of Soil. VÚPOP, Bratislava, Slovakia, 156 p. (In Slovak.)

Fungo, B., Lehmann, J., Kalbitz, K., Tenywa, M., Thiongo, H., Nevfeld, H., 2017. Emissions intensity and carbon stocks of a tropical Ultisol after amendment with Tithonia green manure, urea and biochar. Field Crops Res., 209, 179–188.

Gaida, A. M., Przewloka, B., Gawryjolek, K., 2013. Changes in soil quality associated with tillage system applied. Int. Agrophys., 27, 133–141.

Geisseler, D., Scow, K.M., 2014. Does long-term use of mineral fertilisers affect the soil microbial biomass? Better Crops with Plant Food, 98, 4, 13–15.

Grosbellet, G., Vidal-Beaudel, L., Caubel, V., Charpentier, S., 2011. Improvement of soil structure formation by degradation of coarse organic matter. Geoderma, 162, 27–38.

Gul, S., Whalen, J.K., Thomas, B.W., Sanchdeva, V., Deng, H.Y., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. Agric. Ecosys. Environ., 206, 46–59.

Gupta, V.V., Germida, J.J., 2015. Soil aggregation: influence on microbial biomass and implications for biological processes. Soil Biol. Biochem., 80, A3–A9.

Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure application on soil organic matter content and soil physical conditions: a review. Nut. Cycl. Agroecosys., 51, 123–137.

Horák, J., 2015. Testing biochar as a possible way to ameliorate slightly acidic soil at the research field located in the Danubian Lowland. Acta Hort. Regiotec., 18, 20–24.

Hrivňáková, K., Makovníková, J., Barančíková, G., Bezák, P., Bezáková, Z., Dodok, R., Grečo, V., Chlpík, J., Kobza, J., Lištjak, M., Mališ, J., Píš, V., Schlosserová, J., Slávik, O., Styk, J., Širáň, M., 2011. Uniform Methods of Soil Analyses. VÚPOP, Bratislava, Slovakia, 112 p. (In Slovak.)

Hu, F., Xu, Ch., Li, H., Li, S., Yu, Z., Li, Y., He, X., 2015. Particles interaction forces and their effects on soil aggregates breakdown. Soil Till. Res., 147, 1–9.

Igaz, D., Šimanský, V., Horák, J., Kondrlová, E., Domanová, J., Rodný, M., Buchkina, N.P., 2018. Can a single dose of biochar affect selected soil physical and chemical characteristics? J. Hydrol. Hydromech., 66, 421–428.

IUSS Working Group WRB, 2015. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106, FAO, Rome., 112 p.

Jien, S.H., Wang, Ch.Ch., Lee, T.Y., 2015. Stabilization of organic matter by biochar application in compost–amended soils with contrasting pH values and textures. Sustainability, 7, 13317–13333.

Jindo, K., Sonoki, T., Matsumoto, K., Canellas, L., Roig, A., Sanchez–Monedero, M.A., 2016. Influence of biochar addition on the humic substances of composting manures. Waste Management, 49, 545–552.

Jozefaciuk, G., Czachor, H., 2014. Impact of organic matter, iron oxides, alumina, silica and drying on mechanical and water stability of artificial soil aggregates. Assessment of new method to study water stability. Geoderma, 221–222, 1–10.

Juriga, M., Šimanský, V., 2019. Effects of biochar and its reapplication on soil pH and sorption properties of silt loam Haplic Luvisol. Acta Hort. Regiotec., 22, 66–71.

Juriga, M., Šimanský, V., Horák, J., Kondrlová, E., Igaz, D., Polláková, N., Buchkina, N., Balashov, E., 2018. The effect of different rates of biochar and biochar in combination with N fertilizer on the parameters of soil organic matter and soil structure. J. Ecol. Engin., 19, 153–161.

Kondrlová, E., Horák, J., Igaz, D., Dobiášová, D., 2017. The possibility of using digital images in assessment of plant canopy development and weed spread. Acta Hort. Regiotec., 20, 36–40.

Kopittke, P.M., Menzies, N.W., Wang, P., McKenwa, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environ. Internat., 132, 105–109.

Leelamanie, D.A.L., Karube, J., 2014. Water stable aggregates of Japanese Andisol as affected by hydrophobicity and drying temperature. J. Hydrol. Hydromech., 62, 97–100.

Leelamanie, D.A.L., Manawardana, C.U., 2019. Soil hydrophysical properties as affected by solid waste compost amendments: seasonal and short-term effects in an Ultisol. J. Hydrol. Hydromech., 67, 232–239.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems – a review. Mitig. Adapt. Strat. Glob. Change, 11, 395–419.

Lehmann, J., Rillig, M.C., Thies J., Masiell, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota, A review. Soil Biol. Biochem., 43, 1812–1836.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J., 70, 1719–1730.

Liang, G., Wang, X., Zhou, X., Song, D., Zhang, X., 2015. Characteristic of maize biochar with different pyrolysis temperatures and its effect on organic carbon, nitrogen and enzymatic activities after addition to fluvo-aquic soil. Sci. Total Environ., 538, 137–144.

Liu, Z., Chen, X., Jing, Y., Li, Q., Zhang, J., Huang, Q., 2014. Effects of biochar amendment on rapessed red soil. Catena, 123, 45–51.

Loginow, W., Wisniewski, W., Gonet, S.S., Ciescinska, B., 1987. Fractionation of organic carbon based on susceptibility to oxidation. Pol. J. Soil Sci., 20, 47–52.

Lu, W.W., Ding, W., Zhang, J.H., Li, Y., Luo, J.F., Bolan, N., Xie, Z.B., 2014. Biochar suppressed the decomposition of organic carbon in activated sandy loam soil a negative priming effect. Soil Biol. Biochem., 76, 12–21.

Lugato, E., Simonetti, G., Morari, F., Nardi, S., Berti, A., Giardi, L., 2010. Distribution of organic and humic carbon in wet-sieved aggregates of different soil under long-term fertilization experiment. Geoderma, 157, 80–85.

Mierzwa-Hersztek, M., Gondek, K., Kopeć, M., Ukalska-Jaruga, A., 2018. Biochar changes in soil based on quantitative and qualitative humus compounds parameters. Soil Sci. Ann., 69, 234–242.

Moreno–Barriga, F., Díaz, V., Acosta, J.A., Muñoz, M.Á., Faz, Á., Zornoza, R., 2017. Organic matter dynamics soil aggre-

gation and microbial biomass and activity in Technosols created with metalliferous mine residues, biochar and marble waste. Geoderma, 301, 19–29.

Mukherjee, A., Lal, R., 2013. Biochar impact on soil physical properties and greehouse gas emissions. Agronomy, 3, 313–339.

Obia, A., Mulder, J., Martines, V., Conrelissen, G., Borresen, T., 2016. In situ if biochar on aggregation, water retention and porosity in light-textured tropical soil. Soil Till. Res., 155, 35–44.

Onweremadu, E.U., Onyia, V.N., Anikwe, M.A.N., 2007. Carbon and nitrogen distribution in water-stable aggregates under two tillage techniques in Fluvisols of Owerri area, southeastern Nigeria. Soil Till. Res., 97, 195–206.

Paradelo, R. van Oort, F., Chenu, C., 2013. Water-dispersible clay in bare fallow soils after 80 years of continuous fertilizer addition. Geoderma, 200-201, 40–44.

Pires, L.P., Borges, J.A., Cooper, M., Kosa, J.A., Heck, R.J., 2017. Soil structure changes induced by tillage systems. Soil Till. Res., 165, 66–79.

Polláková, N., Šimanský, V., Kravka, M., 2018. The influence of soil organic matter fractions in aggregates stabilization in agricultural and forest soils of selected Slovak and Czech hilly lands. J. Soils Sediments, 18, 2790–2800.

Ren, X., Yuan, X., Sun, W., 2018. Dynamic changes in atrazine and phenauthrene sorption behaviors during the aging of biochar in soils. Environ. Sci. Poll. Res., 25, 81–90.

Rząsa, S., Owczarzak, W., 2004. Structure of mineral soils. Wyd. Akademii Rolniczej im. Augusta Cieszkoweskiego, Poznañ, 393 p. (In Polish.)

Scott, H.D., 2000. Soil Physics: Agriculture and Environmental Application. Wiley–Blackwell, 421 p.

Shackley, S., Ruysschaert, G., Zwart, K., Glaser, B., 2016. Biochar in European Soils and Agriculture, Science and Practice. Routledge, London, New York, 301 p.

Šimanský, V., 2013. Soil organic matter in water-stable aggregates under different soil management practices in a productive vineyard. Arch. Agron. Soil Sci., 59, 1207–1214.

Šimanský, V., Bajčan, D., 2014. Stability of aggregates and their ability of carbon sequestration. Soil Water Res., 9, 111–118.

Šimanský, V., Horák, J., Igaz, D., Jonczak, J., Markiewicz, M., Felber, R., Rizhiya, E.Y., Lukac, M., 2016. How dose of biochar and biochar with nitrogen can improve the parameters of soil organic matter and soil structure? Biologia, 71, 989–995.

Šimanský, V., Horák, J., Igaz, D., Balashov, E., Jonczak, J., 2018a. Biochar and biochar with N fertilizer as a potential tool for improving soil sorption of nutrients. J. Soils Sediments, 18, 1432–1440.

Šimanský, V., Igaz, D., Horák, J., Šurda, P., Kolenčík, M., Buchkina, N.P., Uzarowicz, L., Juriga, M., Šrank, D., Pauková, Ž., 2018b. Response of soil organic matter and water-stable aggregates to different biochar treatments including nitrogen fertilization. J. Hydrol. Hydromech., 66, pp. 429–436.

Sing, B.P., Cowie, A.L., 2014. Long–term influence of biochar on native organic carbon mineralisation in a low-carbon clayey soil. Sci. Reports, 132, 82–84.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter Dynamics. Soil Till. Res., 79, 7–31.

Spaccini, R., Piccolo, A., Conte, P., Habermanuer, G., Gerzaber, M.M., 2002. Increased soil organic carbon sequestration through hydrophobic protection by humic substances. Soil Biol. Biochem., 34, 1839–1851.

Shukla, M.K., 2014. Soil Physics: An Introduction. CRC Press, Taylor and Francis Group, Boca Raton, London, New York, 458 p.

Spokas, K., Yargigoglu, E.N., Sadasivam, B.Y., Reddy, K.R., 2015. Physical and chemical characterization of waste wood derived biochars. Waste Management, 36, 256–268.

Tisdall, J.M., Oades J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci., 33, 141–163.

Toková, L., Igaz, D., Aydin, E., 2019. Measurement of volumetric water content by gravimetric and time domain reflectometry methods at field experiment with biochar and N fertilizer. Acta Hort. Regiotec., 22, 62–65.

Vitkova, J., Kondrlova, E., Rodny, M., Surda, P., Horak, J., 2017. Analysis of soil water content and crop yield after biochar application in field conditions. Plant Soil Environ., 63, 569–573.

Wang, H., Guan, D., Zhang, R., Chen, Y., Hu, Y., Xiao, L., 2014. Soil aggregates and organic carbon affected by the land use change from rice paddy to vegetable field. Ecol. Engin., 70, 206–211.

Wang, D., Fonte, S.J., Parikh, S.J., Six, J., Scow, K.M., 2017. Biochar additions can enhance soil structure and the physical stabilization of C in aggregates. Geoderma, 303, 110–117.

Wang, Y., Wang, Z.L., Zhang, Q., Hu, N., Li, Z., Lou, Y., Li, Y., Xue, D., Chen, Y., Wu, Ch., Zou, Ch.B., Kuzyakov, Y., 2018. Long-term effects of nitrogen fertilization on aggregation and localization of carbon, nitrogen and microbial activities in soil. Sci. Total Environ., 624, 1131–1139.

Zhao, S., Tan, N., Li, Z., Yang, Y., Zhang, X., Liu, D., Zhang, A., Wang, X., 2017. Varying pyrolysis temperature impacts application effects of biochar on soil labile organic carbon and humic fractions. Appl. Soil Ecol., 116, 399–409.

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